Table 4

Gene no.	Immobilized gene (Gene product)	Primer pair (SEQ ID NO)
1.	Smad3	1, 2
2.	VEGF receptor	3, 4
3.	ACTR	5, 6
4.	N-CoR/SMRT	7, 8
5.	efp	9, 10
6.	c-Myc-1	11, 12
7.	Vitamin D receptor	13, 14
8.	cathepsin G	Commercially available
9.	c-Myc-2	15, 16
10.	Bax	17, 18
11.	JNK1	19, 20
12.	p38	21, 22
13.	TRIP 1	23, 24
14.	ARA 70	25, 26
15.	insulin receptor	27, 28
16.	NGF receptor	Commercially available
17.	PDGF receptor	29, 30
18.	CSF1 receptor-1	Commercially available
19.	CSF1 receptor-2	31, 32
20.	FGF receptor	33, 34
21.	p38 gamma	35, 36
22.	Bcl-X	37, 38
23.	c-Myc-3	39, 40
24.	pS2 protein	41, 42
25.	lactoferrin	43, 44
26.	RIP 140	45, 46
27.	TIF2	47, 48
28.	JNK2	49, 50
29.	Bax delta	51, 52
30.	BMK-1	53, 54
31.	BMK-2	55, 56
32.	Src-1	57, 58
33.	p300/CBP	59, 60
34.	β-actin (positive control)	61, 62
35.	pBR 322 (negative control)	

cDNA fragments for these genes of about 100 b to about 1 kb were prepared using the respective primer pairs as indicated in Table 4 according to the method as

described in Example 1, and spotted to prepare a DNA array. Genes for cathepsin G, NGF receptor and CSF1 receptor were amplified using primers for the respective genes contained in Human UniGene DNA set (Research Genetics).

(2) Examination of influence by endocrine disruptor

Influences by treatment with various endocrine disruptors for 2 or 24 hours on cultured cells were examined.

Treatment for 2 hours: Human breast cancer MCF-7 cells were grown in DME medium containing 10% fetal bovine serum (FBS). After trypsinization, 2 x 10^6 cells were placed in a 10-cm dish. The cells were cultured for 24 hours in DME medium containing 5% fetal bovine serum from which steroid hormones had been removed by treatment with activated carbon-dextran. After removing the medium, the cells were cultured for 2 hour in the same medium containing 10 nM 17-β estradiol (E_2), 10 nM diethylstilbestrol (DES) or 5 μM bisphenol-A (BisA). As a control, cells which were cultured in the absence of such a chemical substance were similarly prepared. The treated cells were recovered, and total RNAs were extracted as described in Example 2(1).

Treatment for 24 hours: Human breast cancer MCF-7 cells were grown in DME medium containing 10% fetal bovine

serum (FBS). After trypsinization, 2 x 10^6 cells were placed in a 10-cm culture dish. The cells were incubated for 24 hours in the same medium. After removing the medium, the cells were cultured for 24 hours in DME medium containing 5% human serum from which steroid hormones had been removed by treatment with activated carbon-dextran in the presence of 10 nM 17-β estradiol (E_2), 10 nM diethylstilbestrol (DES) or 5 μM bisphenol-A (BisA). As a control, cells which were cultured in the absence of such a chemical substance were similarly prepared. The treated cells were recovered, and total RNAs were extracted as described in Example 2(1).

(3) The total RNAs as prepared in (2) above were treated with DNase. Reaction mixtures each containing about 100 to about 300 μg of the total RNA, 10 μl of 10 x AMV buffer (Life Science) and 10 U of DNaseI (Takara Shuzo) in a volume of 12 μl were prepared, incubated at 37°C for 10 minutes, extracted twice with phenol/chloroform, and then subjected to ethanol precipitation. The concentrations of the resulting total RNAs were determined using portions thereof.

(4) Reverse transcription reaction was carried out using one of the total RNAs as prepared in (3) above. The composition of the reaction mixture was as follows.

Reaction mixture A: about 130 μg of the total RNA,